PCT

WORLD INTELLECTUA
Interna

INTERNATIONAL APPLICATION PUBLISHED U

(51) International Patent Classification 6 :
C09C

A2

WO 9602594A2

(43) International Publication Date: 1 February 1996 (01.02.96)

(21) International Application Number: PCT/IB95/00535

(22) International Filing Date: 3 July 1995 (03.07.95)

(30) Priority Data:
08/277,609 20 July 1994 (20.07.94) US

(71) Applicant: INDUSTRIAL ORGANICA, S.A. DE C.V. [MX/MX]; Avenida Almazan 100, Colonia Topo Chico, Monterrey, Nuevo León 64620 (MX).

(72) Inventors: TORRES-CARDONA, Mario-David; Araucaria 128, Fraccionamiento Paseo de Los Angeles, San Nicolas de los Garza, Nuevo León 66470 (MX). TORRES-QUIROGA, Jose; Tajin 105, Colonia Valle de San Angel, San Pedro Garza Garcia, Nuevo León 66290 (MX).

(81) Designated States: AM, AU, BB, BG, BR, BY, CA, CN, CZ, DK, EE, FI, GB, GE, HU, IS, JP, KG, KP, KR, KZ, LK, LR, LT, LV, MD, MG, MN, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TM, TT, UA, UG, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG).

**Published**
*Without international search report and to be republished upon receipt of that report.*

(54) Title: PROCESS FOR THE ISOMERIZATION OF LUTEIN

## (57) Abstract

A process to obtain a product with a high zeaxanthin content, to be used mainly as an ingredient in poultry feed to enhance the pigmentation of broiler chickens and egg yolk, by reacting at a controlled temperature and pressure, marigold *Tagetes erecta L.* meal or its oleoresin, or formulations containing lutein, with strongly alkaline aqueous solutions under controlled conditions, to isomerize the lutein into zeaxanthin in a product with a higher pigmenting activity.

*FOR THE PURPOSES OF INFORMATION ONLY*

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

| | | | | | |
|---|---|---|---|---|---|
| AT | Austria | GB | United Kingdom | MR | Mauritania |
| AU | Australia | GE | Georgia | MW | Malawi |
| BB | Barbados | GN | Guinea | NE | Niger |
| BE | Belgium | GR | Greece | NL | Netherlands |
| BF | Burkina Faso | HU | Hungary | NO | Norway |
| BG | Bulgaria | IE | Ireland | NZ | New Zealand |
| BJ | Benin | IT | Italy | PL | Poland |
| BR | Brazil | JP | Japan | PT | Portugal |
| BY | Belarus | KE | Kenya | RO | Romania |
| CA | Canada | KG | Kyrgystan | RU | Russian Federation |
| CF | Central African Republic | KP | Democratic People's Republic of Korea | SD | Sudan |
| CG | Congo | KR | Republic of Korea | SE | Sweden |
| CH | Switzerland | KZ | Kazakhstan | SI | Slovenia |
| CI | Côte d'Ivoire | LI | Liechtenstein | SK | Slovakia |
| CM | Cameroon | LK | Sri Lanka | SN | Senegal |
| CN | China | LU | Luxembourg | TD | Chad |
| CS | Czechoslovakia | LV | Latvia | TG | Togo |
| CZ | Czech Republic | MC | Monaco | TJ | Tajikistan |
| DE | Germany | MD | Republic of Moldova | TT | Trinidad and Tobago |
| DK | Denmark | MG | Madagascar | UA | Ukraine |
| ES | Spain | ML | Mali | US | United States of America |
| FI | Finland | MN | Mongolia | UZ | Uzbekistan |
| FR | France | | | VN | Viet Nam |
| GA | Gabon | | | | |

# PROCESS FOR THE ISOMERIZATION OF LUTEIN.

## BACKGROUND OF THE INVENTION

An adequate pigmentation is a major concern of the poultry industry. Not only it is synonymous of good healthy birds, but at the same time it is also important marketing strategy in many cultures. Very frequently, poultry producers make considerable efforts in order to obtain an attractively pigmented bird-carcass that appeals to the eye of the consumer.

To satisfy the market preferences, chicken producers have traditionally supplemented the poultry diets with carotenoid (particularly hydroxicarotenoids or xanthophylls) containing extracts or meals of plant origin that are responsible for the desired yellow-orange color in the broiler chickens and in egg yolks. A widely used source of xanthophylls is marigold meal and its extract. The carotenoids from marigold contain approximately from 85 to 90% of lutein ($\beta,\epsilon$ - caroten- 3,3' - diol) and from 2 to 4 % of zeaxanthin ($\beta$, $\beta$ - caroten 3, 3' - diol)(F.W. Quackenbush, 1972. J.AOAC, 55 (3), 617-21). These hydroxicarotenoids occur in the plant as esters of fatty acids, mainly palmitic, myristic and stearic (W. Gau. 1983, J. Chromatogr. 262, 277-84).

However, it has been proved that a more efficient pigmentation is obtained when the xanthophylls are in their free form (Fletcher, D.L. 1986, Poult. Sci., 65(9), 1708-

14) after the ester link has been broken by means of a saponification process. As a matter of fact, most of the pigment producers carry on a saponification process in order to improve the bioavailability of the xanthophylls.

Furthermore, it has been demonstrated that a synergistic effect occurs when carotenoids like lutein and zeaxanthin are mixed with other natural or synthetic pigments for broiler chicken and egg yolk pigmentation (US Pat. No. 3,539,686 of November 10, 1970 to Ralph Rosenberg).

Different methods have been practiced in the past decades for the preparation of xanthophyll concentrates from marigold meal. As an example, in the US Patent No. 3,523,138 from August 4, 1970. issued to Eastman Kodak Co., a process is described wherein the marigold meal is reacted with an alcoholic alkali solution. After saponification the mixture is diluted with water and the xanthophylls are separated by solvent extraction by means of water insoluble solvent like isopropyl ether.

US Patent No. 3,535,426 dated October 1970, issued also to Eastman Kodak Co., describes a method to stabilize xanthophylls by adding an antioxidant ethoxyquin (6.ethoxy-2,2,4-trimethil-1,2-hydroquinoline) and fat, and heating the mixture afterwards. The product obtained is very stable and does not degrade appreciably.

Also Ger. Offen. 2,535,963 dated March 4, 1976, issued to CPC International Inc., describes a process to stabilize xanthophylls by partial saponification using a solution of

potassium hydroxide in methanol.

US Patent No. 3,783,099 dated >January 1st 1974 issued to Ralston Purina Co., a process is described where an enzymatic hydrolysis of the cellulosic material of the marigold meal, improves the extraction of the xanthophylls with a non polar solvent.

Recently some fermentative processes have been described wherein the reproduction of improved strains of Flavobacterium Multivorum result in the obtainment of zeaxanthin extracts with a pigment activity 2 to 3 times higher than that of marigold extracts, and are suggested as an alternative to naturally occurring carotenoids; as described on PCT Int. Appln. WO 91 03,571 dated March 21, 1991, issued to Applied Biotechnology, Inc.

Traditional sources of zeaxanthin have been the extracts and meals of yellow corn and yellow corn gluten.

Years ago, Karrer and Jucker in 1947 (Helv. Chim. Acta. 30,266-7) obtained zeaxanthin by isomerizing lutein in a reaction catalyzed with sodium ethoxide in the presence of ethanol and benzene. A.G. Andrews, also in 1947 (Acta Chem. Scand. B 28 No. 1) obtained zeaxanthin from lutein using potassium methoxide in the presence of methanol and dimethylsulfoxide. In both procedures a poor yield was obtained resulting in a great loss of pigment. The economy of such processes is inadequate for industrial purposes. However, they demonstrated the feasibility to isomerize lutein into zeaxanthin.

In other words, isomerization of lutein into

zeaxanthin has been carried out in academic research basis in a catalytic organic phase in the presence of solvents with a very strong alcoholic alkaline solutions which could cause a very violent exothermic reaction in the presence of water or humidity.

This process is related with the isomerization of lutein into zeaxanthin in a non catalytic aqueous phase which does not needs the presence of solvents.

In the marigolds meal extracts available in the market, zeaxanthin represents only from 4 to 6% of the total xanthophill content. Since the pigment activity of this carotenoid is higher than that of lutein, applicants have developed a process where a higher. concentration of zeaxanthin is obtained, and hence the pigment efficiency of the extract containing this carotenoid is improved.

## SUMMARY OF THE INVENTION

It is therefore a main object of the present invention, to provide a process to isomerize lutein into zeaxanthin in a non catalytic aqueous phase with a strongly alkaline aqueous solutions and under controlled conditions which does not needs the presence of solvents.

It is also a main object of the invention, to provide a process to isomerize lutein into zeaxanthin from marigold (Tagetes erecta L.) meal, its oleoresin or formulations containing lutein.

It is still a main object of the invention, to provide a process to isomerize lutein into zeaxanthin to

produce a product with a high zeaxanthin content which can be used to pigment broiler chickens and egg yolks, as a pigmenting ingredient in aquaculture and as an ingredient for food consumption.

It is a further main object of the present invention, to provide a process to isomerize lutein into zeaxanthin, of the above disclosed nature, wherein the presence of the zeaxanthin in the obtained product represents from 5 to 50% of the total xanthophylls, usually from 8 to 30%, and preferably from 10 to 20%.

It is an additional object of the present invention, to provide a process to isomerize lutein into zeaxanthin, of the above disclosed nature, which is safely, commercially and economically viable because it is carried out in a non catalytic aqueous phase which consequently does not needs organic solvents.

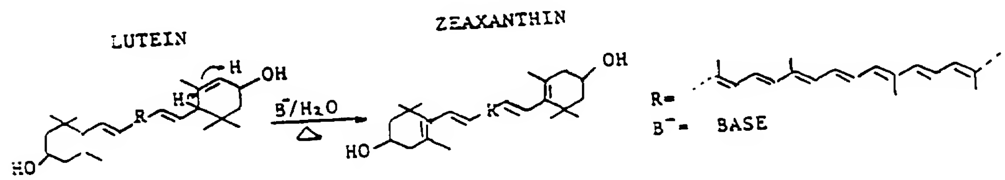
These and other objects and advantages of the present invention will be apparent to the persons skilled in the art, from the following description of the specific embodiments of the invention, represented by the performed examples.

## DETAILED DESCRIPTION OF THE INVENTION

The standard method of analysis accepted in the industry to quantify total xanthophylls in marigold meals and its extracts is described in the "AOAC Official Method of Analysis" (edited by Sidney Williams, Fourteenth edition, pages 835-6, 1984).

The HPLC methods to separate, identify and quantify lutein and zeaxanthin are described in the work of Yitzhak Itta (J. Agric. Food. Chem., 1993,41, 899-901) and of Lane C. Sander (Anal. Chem., 1994, 66, 1667-1674).

The process occurs according to the following mechanism:




The extract that contains the lutein can be maintained at any temperature from 25 to 180°C, preferably from 70 to 110°C. The slow addition of strongly alkaline aqueous solution is followed, wherein the alkali can be sodium hydroxide (NaOH), potassium hydroxide (KOH), calcium hydroxide (Ca(OH)2), Sodium carbonate (Na2CO3), ammonium hydroxide (NH4OH), or any other similar compound or their mixtures. Organic basis like ethylamine, ethanolamine, morpholine or others of similar nature, or their mixtures can be used..

The alkali concentration in the aqueous solution can be from 5 to 50% by weight, preferably between 25% and 45% by weight. The amount of alkali can be in the range of 0.1 to 1.0 parts for one part of marigold meal extract, or more preferably from 0.3 to 0.7 parts of alkali to one part of the lutein containing concentrate.

The reaction time is strongly dependent on the temperature and amount of alkali used, but can be from 5 to

96 hours, usually from 16 to 36 hours, and preferably from 30 to 48 hours..

The process can be carried out in a reactor or container at atmospheric pressure but if shorter reaction times are desired the pressure can be increased from 5 to 150 psig, preferably from 20 to 50 psig. By this means the reaction time can be from 5 minutes to 12 hours, usually from 3 to 8 hours. It is desired to keep an inert atmosphere using either steam, nitrogen, carbon dioxide or similar compounds or their mixtures. If desired, the reaction can be carried out at a reduced pressure to avoid xanthophyll degradation.

Typical examples are illustrated on table 1, indicating some of the results that have been obtained, these are examples only, and should not be interpreted as limiting the scope of this invention.

Table 1

| | | | | |
|---|---|---|---|---|
| Lot No. | 1 | 2 | 3 | 4 |
| Alkali | KOH | NaOH | Na2CO3 | KOH |
| Alkali/Extract, w/w | 0.3 | 1.0 | 0.5 | 0.5 |
| Temperature °C | 80 | 90 | 105 | 95 |
| Pressure, atm. | 0.5 | 1.0 | 2.0 | 1.0 |
| Time, Hr. | 48 | 30 | 3 | 36 |
| ¬Initial Lutein % | 85.5 | 85.5 | 85.5 | 85.5 |
| Final lutein % | 68.9 | 66.2 | 60.8 | 68.3 |

| | | | | |
|---|---|---|---|---|
| —Initial Zeaxanthin % | 4.5 | 4,5 | 4.5 | 4.5 |
| Final zeaxanthin % | 16.1 | 18.7 | 24.0 | 15.8 |

¬,−: the initial extract contents 97 grams of total xanthophylls per kilo of extract, and on this value are calculated the percentages.

The product obtained can be formulated as a water dispersion by means of suitable emulsifiers or can be dispersed on a carrier to make a premix or can be further concentrated by solvent extraction.

WHAT WE CLAIM IS:

1. A process to isomerize lutein into zeaxanthin, comprising treating a lutein reaction substrate from any natural source or synthetic origin or a mixture thereof, with a strongly aqueous alkaline solution under controlled conditions of temperature and pressure and for a length of time that depends on the degree of the desired isomerization.

2. The process according to claim 1, in which the lutein substrate contains lutein in a hydrolyzed or sterified form.

3. The process according to claim 1, in which the lutein reaction substrate can be an extract or concentrate obtained from marigold flowers (Tagetes erecta L.) or marigold meal.

4. The process according to claim 1, in which the lutein reaction substrate can be any natural or synthetic material containing xanthophylls, mainly free lutein in any proportion; preferably yellow corn, yellow corn gluten or its extracts or mixtures.

5. The process according to claim 1, wherein the lutein reaction substrate can be any material either natural or synthetic that contains xanthophylls, and mainly lutein in a naturally occurring sterified form, in any proportion.

6. The process according to claim 1 in which the aqueous alkali solutions are aqueous solutions which can contain from 5 to 50% by weight of alkali.

7. The process according to claims 1 and 6, in which the

alkali solutions can be alkali saturated or oversaturated solutions.

8. The process according to claims 1, 6 and 7, in which the alkali can be potassium hydroxide, sodium hydroxide, sodium carbonate, ammonium hydroxide, ammonia and similar compounds or their mixtures, or in its anhydrous form.

9. The process according to claims 1 and 6 to 8, in which the alkali can be an organic base like ethanolamine, ethylamine, morpholine, and similar compounds or mixtures thereof.

10. The process according to claims 1 and 6 to 9, in which the ratio by weight of alkali to the lutein containing substrate, can be 0.05:5, usually 0.1:3 and preferably 0.2:1.

11. The process according to claim 1, in which the reaction time of the alkaline solution and the lutein containing substrate can be from 5 minutes to 96 hours.

12. The process according to claim 1, in which the reaction is catalyzed by enzymatic means, like a catalase or an isomerase.

13. The process according to claim 1, in which the isomerization of lutein can be carried out at pressures of 5 to 150 psig, and usually at atmospheric pressure.

14. The process according to claims 1 and 13, in which the isomerization reaction can be carried out at a reduced pressure.

15. A process according to claim 1, in which the reaction can be carried out under an inert atmosphere

conditions like carbon dioxide, nitrogen or compounds of similar nature or their mixtures.

16. The process according to claim 1, in which the reaction temperature can be from 25 to 180°C and preferably from 70 to 110°C.

17. The process according to claim 1, in which the isomerization reaction time is improved by means of ultrasonic waves.

18. The process according to claim 1, in which, in the product obtained, the zeaxanthin represents from 5 to 50% of the total xanthophylls, usually from 8 to 30%, and preferably from 10 to 20%.

19. The process according to claim 1, in which the product obtained or its formulations, can be used to pigment broiler chickens and egg yolks.

20. The process according to claim 1, in which the product obtained or its formulations, can be used as pigmenting ingredient in aquaculture.

21. The process according to claim 1, in which the product obtained or its formulations, can be used as an ingredient for food consumption.

22. A lutein isomerized substrate having a zeaxanthin content from 5 to 50% of the total xanthophylls, usually from 8 to 30%, and preferably from 10 to 20%, produced by isomerizing lutein from any natural source of synthetic origin or a mixture thereof, with a strongly aqueous alkaline solution under controlled conditions of temperature and pressure and for a length of time that

depends on the degree of the desired isomerization.

23. The process according to claim 1, in which the lutein reaction substrate is any natural material containing xanthophylls selected from the group consisting of yellow corn, yellow corn gluten, or its extracts or mixtures.

24. The process according to claim 1, in which the ratio, by weight, of alkali to the lutein containing substrate is about 0.1:3.

25. The process according to claim 1, in which the ratio, by weight, of alkali to the lutein containing substrate is about 0.2:1.

26. The process according to claim 16, in which the reaction temperature is from about 70°C to about 110°C.

27. The process according to claim 1, in which in the product obtained the zeaxanthin represents from about 8% to about 30% of the total xanthophylls.

28. The process according to claim 1, in which in the product obtained the zeaxanthin represents from about 10 to about 20% of the total xanthophylls.

29. A lutein isomerized substrate according to claim 22, having a zeaxanthin content of from about 8% to about 30% of the total xanthophylls.

30. A lutein isomerized substrate according to claim 22, having a zeaxanthin content of from about 10% to about 20% of the total xanthophylls.